

Interactive effects of elevated CO₂, temperature and nitrogen on photosynthesis of wheat grown under temperature gradient tunnels

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Summary

The effects of increased CO₂, temperature and nitrogen on leaf photosynthesis of wheat were investigated in two field experiments under temperature gradient tunnels in a Mediterranean environment. Ambient and 700 μmol mol⁻¹ CO₂, ambient and 4 °C warmer temperatures, and 80 and 120 kg nitrogen ha⁻¹ were compared. Although rising CO₂ concentrations increased photosynthesis, measurements at the same CO₂ concentration showed decreased photosynthesis and stomatal conductance in plants grown at elevated CO₂. Elevated growth CO₂ decreased photosynthesis for any given value of intercellular CO₂ concentration. Downward acclimation of photosynthesis was decreased at temperatures 4 °C above ambient and high nitrogen supply, under both photorespiratory and non-photorespiratory measurement conditions. Growth in elevated CO₂ decreased the quantum yield of Photosystem II (PSII) electron transport and the efficiency of energy capture by open PSII centres. At later stages of leaf growth, warm temperatures decreased maximal photochemical efficiency (F_v/F_m) at low, but not at high nitrogen supply. F_v/F_m increased with nitrogen application, although the quantum yield of electron transport in the light remained unchanged.

Key-words: *Triticum aestivum*, chlorophyll fluorescence, climate change, elevated CO₂, elevated temperature, nitrogen, photosynthetic acclimation; stomatal conductance, Rubisco.

Abbreviations: AC and EC, ambient and elevated growth CO₂, respectively; F_o , F_m , minimal and maximal fluorescence levels in the dark adapted state, respectively; F_o' , F_m' , F_s , minimal, maximal and steady-state fluorescence in the light adapted state, respectively; Φ_{II} , quantum yield of Photosystem II electron transport; HN, high nitrogen supply; LN, low nitrogen supply; PSII, Photosystem II; qP, photochemical quenching coefficient; Rubisco, ribulose-1, 5-bisphosphate carboxylase oxygenase; T and T+4, ambient and ambient+4 °C growth temperature, respectively.

Introduction

After prolonged growth with CO₂ enrichment, plants are frequently incapable of maintaining the initial stimulation of photosynthesis, which according to Rogers and Humphries (2000) is entirely attributable to a decrease in maximal velocity of Rubisco carboxylation. It has been proposed that photosynthesis acclimation is due to a sugar mediated repression of gene expression for Rubisco and other proteins involved in photosynthesis (Sheen 1990, Stitt 1991). However, with a high nitrogen supply sugars increase at elevated CO₂ but no acclimation of photosynthesis or decrease of transcripts of the Benson-Calvin cycle is observed (Geiger et al. 1999). The decrease in photosynthesis and Rubisco is usually more marked in nitrogen limited than in well fertilized plants (Stitt and Krapp 1999). Notwithstanding, when plant growth rate is adjusted to a low nitrogen supply, so that there is no decrease of leaf nitrogen content, no acclimation of photosynthesis occurs (Farage et al 1998). Moreover, under conditions where nitrogen deficiency reduced leaf area, photosynthesis and sugar levels and increased biomass allocation to non-photosynthetic tissues, the magnitude of downward acclimation of photosynthesis to elevated CO₂ was greater with high compared to low nitrogen supply (Jifon and Wolfe 2002). Therefore, the acclimation response of photosynthesis to elevated CO₂ seems to depend on nitrogen in a manner regulated by the whole plant source: sink balance.

Plants growing for a long term under elevated CO₂ also experience a downward acclimation of stomatal conductance (Bunce 2001, Lodge et al. 2001) and a decrease of stomatal sensitivity to low intercellular CO₂ (Santrucek and Sage 1996, Lodge et al. 2001). The stomatal limitation of photosynthesis increases under elevated as compared to ambient CO₂ when measured at the same CO₂ concentration (Lodge et al. 2001). Whether stomata acclimate in parallel with, or independently of any mesophyll photosynthetic acclimation is still under discussion, although some recent studies suggest that stomatal acclimation is unrelated to the degree of photosynthetic acclimation (Lodge et al. 2001, Medlyn et al 2001). Down regulation of stomatal conductance is greater with a lower vapour pressure deficit and with higher temperatures (Bunce 2000 c); We (unpublished results) have found that stomatal limitation of photosynthesis under elevated CO₂ in a Mediterranean semiarid climate is high compared with data reported by others (Hogan et al. 1991, Lodge et al. 2001). The role of stomata in the response of carbon assimilation to long-term growth under

elevated CO₂ may, therefore, vary among environments with contrasting air temperature and humidity.

Acclimation to elevated CO₂ decreased the light harvesting chlorophyll protein contents and the capacity for light saturated photosynthesis without decreasing the quantum efficiency of CO₂ fixation (Osborne et al. 1997). In contrast, the maximal quantum efficiency and the quantum yield of Photosystem II (PSII) electron transport decreased in elevated growth CO₂ in well irrigated plants (Roden and Ball 1996 a) and under drought conditions (Scarascia-Mugnozza et al. 1996), as well as at temperatures of 45 °C (Roden and Ball 1996 b). It has been shown that elevated CO₂ can cause both decreases and increases in light use in photochemistry, depending on seasonal changes in limitations on carbon metabolism (Hymus et al. 1999). While these effects were associated with changes in photochemical quenching and not in the efficiency of energy transfer in the antenna (Hymus et al. 1999), with nitrogen limitation this efficiency was significantly smaller in elevated CO₂ (Hymus et al. 2001).

Photosynthesis models predict an increase in photosynthesis as temperature increases to an optimum, followed by a decrease with warmer temperatures (Long 1991). A rise in growth temperature increases this optimum value for photosynthesis (Yamasaki et al. 2002, Turnbull et al. 2002). Moreover, high night temperatures increase photosynthetic capacity during the light period and decrease pre-dawn carbohydrate levels in association with an increase in dark respiration (Turnbull et al. 2002). Models also predict greater photosynthesis stimulation by CO₂ as temperature increases, because the decrease in photorespiration at elevated CO₂ displaces the optimum temperature to higher values (Long 1991). This stimulation of the response to temperature caused by CO₂ enrichment is altered, however, through acclimation to growth temperature (Hikosaka et al. 1999, Bunce 2000 a, Ziska 2001), which can modify Rubisco specificity for CO₂ (Bunce 2000 b), or alter the potential rate of electron transport and the maximal velocity of Ribulose-1,5-bisphosphate saturated carboxylation (Ziska 2001).

This two-year study was conducted to assess the interactive effects of elevated CO₂ and above ambient temperatures on photosynthesis of field crops of wheat, under Mediterranean conditions of limiting water and warm temperatures. Two wheat varieties known to differ in earliness were compared the first year. Effects of nitrogen supply on the CO₂-temperature interaction were studied in a second experiment. The aim was to investigate, firstly, whether acclimation of

photosynthesis to elevated CO₂ varies with growth temperature and nitrogen supply, distinguishing between stomatal and non-stomatal factors in the regulation of carbon assimilation, and secondly, whether the increases in CO₂ and temperature, at varying nitrogen supply, affect the photochemical efficiency, or only the carboxylation capacity. The study was performed in the field under temperature gradient tunnels – tracking the diurnal and seasonal fluctuations of temperature – in a Mediterranean environment with a water supply equivalent to the average rainfall during the growth season. Diurnal and developmental changes in Rubisco control by CO₂, temperature and nitrogen in the second year have been reported previously (Pérez et al. 2004).

Material and Methods

Experimental setup

Spring wheats (*Triticum aestivum* L.) cvs. Alcazar and Rinconada in a first experiment and only Alcazar in a second, were sown in a clay sand soil at a density of 180 kg ha⁻¹ and 0.13 m row spacing on 17 and 13 February in the first and second year, respectively. The two varieties were sown in adjacent 3 m strips in the first year. Before sowing, N (as NH₄NO₃)-P-K fertilizer complexes with roughly the same proportion of N to P and K were applied the two years (137, 60 and 60 kg ha⁻¹, respectively in the first year, and 80, 40 and 40 kg ha⁻¹, respectively, in the second). The crops were watered weekly through a drip irrigation system providing amounts of water equivalent to the average rainfall in this area during the period of the experiment (198 mm between February and June). The experiment was carried out at the IRNASA farm at Salamanca (41 °N, 800 m above sea level).

The crops were covered with temperature gradient tunnels on 21 April and 23 March in the first and second year, respectively. In the first year technical problems delayed tunnel set up and in the second they were installed in March, rather than at sowing, to select crop areas with a uniform plant cover. The tunnels (9 m long, 2.2 m wide, and 1.7 m high at the ridge) were adapted from Rawson et al. (1995) and are described elsewhere (Pérez et al. 2004). The study was conducted in two tunnels, one kept at the ambient air CO₂ concentration (AC) and another at 700 µmol mol⁻¹ (EC) during the light hours. Since there appears to be no, or only small positive (Davey et al. 2004) direct effects of growth CO₂ on leaf dark respiration, the reports in the literature appearing to be artefacts (Jahnke and Krewitt 2002), lack of CO₂ enrichment during the night is probably irrelevant. The temperature difference between the extreme modules in a tunnel was set at 4 °C (T and T+4 temperatures). In the first year, each of the longitudinal halves of the tunnels covered one variety. Additional nitrogen (40 kg ha⁻¹) was applied to one of these halves in the second year 34 days after sowing, and so two levels of this nutrient (80 and 120 kg ha⁻¹, LN and HN, respectively) were compared. Measurements were repeated in four consecutive sections within the module halves. The diurnal courses of air temperature, relative humidity and CO₂ concentration within the tunnels for the second experiment have been shown elsewhere (Pérez et al. 2004); similar environmental conditions were recorded in the first experiment.

Gas exchange measurements

At the beginning of anthesis (22 May; anthesis occurred about 3 days later with T than with T+4) in the first year, and on day 2 after the start of ear emergence (21 May; ear emergence was advanced about 3 days by T+4) and day 9 after anthesis (3 and 9 June for T+4 and T, respectively) in the second experiment, gas exchange was measured in four flag leaves of each treatment combination. Measurements were performed in clear days between 2 and 8 h after dawn. A replicate leaf from each treatment –with treatments in random order - was measured before the next replicate, so that differences during the day could be included in the replicate effect in the analysis of variance. Leaves were enclosed in a leaf chamber having an 11 cm²-window (PLC(N)-2, ADC, Hoddesdon, Herts., UK) to which were added a new photosynthetically active radiation (PAR) sensor, and an additional humidity sensor (codes LCH-030/S and LCH-032/s, respectively, ADC) to measure inlet and outlet air vapour pressures simultaneously. The chamber temperature (30 ± 1.7 °C during measurements) was measured with the in-built thermistor. The chamber was connected to an infrared gas analyser (LCA-2, ADC) with differential operation in an open system. Ambient air taken 3 m above ground at a place separated from the operator, or air from a gas cylinder containing 700 $\mu\text{mol mol}^{-1}$ CO₂, was humidified by passage through a bubbler, and vapour pressure deficit (1.7 kPa) was adjusted by passing through columns filled with silica gel. Measurements were performed both at 360 and 700 $\mu\text{mol mol}^{-1}$ CO₂ in plants grown at AC and EC. Air flow rate was adjusted to 500 ml min⁻¹ using a mass-flow regulator (ASUM, ADC). During measurements, the chamber was held facing the sun to obtain $1300 \pm 140 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR. The leaf area enclosed within the chamber was calculated by multiplying the chamber length by the average of leaf widths at the two chamber extremes. Carbon assimilation, stomatal conductance (g_s) and CO₂ concentration in the intercellular air spaces (C_i) were calculated according to Long and Hallgreen (1985).

Chlorophyll fluorescence measurements

Eleven days after the beginning of anthesis (3 June) in the first year, the leaves were enclosed in the PLC(N) leaf chamber with circulating air containing 700 $\mu\text{mol CO}_2 \text{mol}^{-1}$ and were illuminated with $110 \mu\text{mol m}^{-2} \text{s}^{-1}$ irradiance. The quantum yield of PSII electron transport : $\Phi_{II} [(F_m' - F_s)/F_m']$, where F_m' is the maximal

fluorescence under irradiance and F_s is the steady-state fluorescence in the light, was measured according to Genty et al. (1989) with a fluorometer (PAM-2000, Walz, Effeltrich, Germany) with the fiber optics through the radiation shield of the leaf chamber at an angle of 60°. Twenty-five days after the beginning of anthesis (17 June) in the first year, and 4 days after the start of ear emergence (22 May) and 7-9 days after anthesis (2 and 10 June for T+4 and T, respectively) in the second year, chlorophyll fluorescence was measured again with ambient CO_2 and $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, using a leaf clip holder (2030B, Walz). Photochemical quenching, $qP [(F_m' - F_s)/(F_m' - F_o')]$, photochemical efficiency of open PSII centres $F_v'/F_m' [(F_m' - F_o')/F_m']$, and Φ_{II} were recorded in the light with saturating light pulses until a steady-state was reached, as described (Martínez-Carrasco et al. 2002). F_o' represents the minimal fluorescence in the dark with a non-photochemical quenching similar to that found in the steady-state in the light. Leaves were darkened for 20 min with a dark leaf clip (DLC8, Walz) before determining the maximal photochemical efficiency, $F_v/F_m [(F_m - F_o)/F_m]$, where F_m and F_o are the maximal and minimum fluorescence in the dark adapted state, respectively. Fluorescence measurements were performed in 4 flag leaves per treatment combination.

Statistical analysis

Analyses of variance were performed as in a nested design according to Snedecor and Cochran (1967), with temperature and nitrogen (or variety in the first year) as a stratum included in CO_2 , and replicates as a stratum included in that for temperature and nitrogen (or variety). The variance ratio for CO_2 was obtained by dividing the mean square for CO_2 (1 degree of freedom) by the nitrogen (or variety) and temperature within CO_2 mean square (6 degrees of freedom). Similarly, the nitrogen (or variety), temperature and interactive effects (1 degree of freedom each) were compared against the replicates within CO_2 -temperature-nitrogen (or variety) mean square (24 degrees of freedom). Where appropriate, measurement CO_2 was included as a further stratum in the analysis. This statistical analysis is further discussed in a preceding paper (Pérez et al. 2004). The analyses were performed with the GenStat 6.2 statistical package. Since the standard error of differences are better estimates of the treatment effects than simply the standard error of means, the least significant differences between treatments (standard error of the difference x Student's t for $P < 0.05$) are shown in figures.

Results

Gas exchange

When measured at the growth CO_2 concentration, photosynthesis of flag leaves was faster in EC plants. However, with the same measurement CO_2 , the response of photosynthesis to growth CO_2 depended on both temperature and nitrogen (Fig. 1). The varieties compared in the first experiment showed no differences in the response of gas exchange to CO_2 or temperature, and thus the results have been averaged. With LN (second experiment) EC decreased photosynthesis measured with a common CO_2 , except 9 days after anthesis at T (Fig. 1 B, C). With HN (both experiments), EC also decreased photosynthesis at T and, in measurements at $700 \mu\text{mol mol}^{-1} \text{CO}_2$ in the first year, also at T+4 (Fig. 1). With this exception, EC did not cause a significant reduction in photosynthesis at T+4, or the decrease was smaller than at T. Thus, under both photorespiratory and decreased photorespiration conditions during measurements, when nitrogen supply was abundant, T+4 generally decreased the inhibition of photosynthesis caused by EC.

With LN (second experiment), T+4 had no significant effects on photosynthesis 2 days after the start of ear emergence (Fig. 1 B), while it increased photosynthesis at AC, but decreased it at EC, 9 days after anthesis (Fig. 1C). With HN, rising temperatures only increased photosynthesis under AC in the first experiment, in measurements at $700 \mu\text{mol mol}^{-1} \text{CO}_2$ (Fig. 1 A), while they always increased photosynthesis at.

HN (second year) had no significant effect on photosynthesis of plants grown under AC 2 days after ear emergence (Fig. 1 B), and increased it 9 days after anthesis (Fig. 1 C) with AC combined with T. Nitrogen also had no effect on photosynthesis of leaves at EC and T, but increased photosynthesis of plants grown at EC and T+4. Thus, an increased nitrogen supply was more beneficial for photosynthesis with the predicted increases of CO_2 combined with warmer temperatures.

The dependence of changes in photosynthesis on diffusion of CO_2 to the sites of carboxylation was assessed by examining the changes in stomatal conductance and intercellular CO_2 concentration of leaves. Generally, EC decreased g_s measured at a common CO_2 (Fig. 2). However, with HN and T+4 2 days after the start of ear emergence (second experiment, Fig. 2 B), as well as with LN and T 9 days after anthesis (Fig. 2 C), EC increased g_s . T+4 increased stomatal conductance of plants grown at EC and HN in all years and sampling dates (Fig. 2), and in plants grown in

EC with LN 2 days after the start of ear emergence (Fig. 2 B), but not 9 days after anthesis (Fig. 2 C). In contrast, T+4 had no effect (with LN) or decreased g_s in plants grown under AC 2 days after the start of ear emergence (second year, Fig. 2 B), although it increased conductance in these plants after anthesis in the two experiments (Fig. 2 A and C). Two days after the start of ear emergence, HN increased stomatal conductance in plants at T, but not at T+4; exceptions were the plants under EC and T+4, where conductance in measurements at $700 \mu\text{mol mol}^{-1} \text{CO}_2$ increased with nitrogen. Nine days after anthesis, HN increased g_s in all treatments, except in plants under EC and T. In association with these changes in stomatal conductance, in the first year EC decreased, and T+4 increased C_i (Fig. 3 A). In the second year, 2 days after the start of ear emergence EC had no significant effects on C_i , while T+4 increased C_i in plants grown at EC (Fig. 3 B). Nine days after anthesis there were no significant changes in C_i (Fig. 3 C). Figure 4 shows the response of carbon assimilation to intercellular CO_2 concentration. It is clear that growth under EC did not decrease photosynthesis by decreasing C_i through reductions in g_s , neither was the inhibition of photosynthesis at elevated CO_2 decreased, when nitrogen supply and temperature were high, as a result of increased C_i .

Chlorophyll fluorescence

Eleven days after anthesis in the first year, the quantum yield of PSII electron transport (ϕ_{II}) measured at $700 \mu\text{mol mol}^{-1} \text{CO}_2$ and $110 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, which are conditions where photosynthesis is limited by Ribulose-1,5-bisphosphate regeneration (Sage 1990), decreased slightly, but significantly at EC (Fig. 5 A). The increase in temperature had no significant effect on ϕ_{II} under these conditions.

Chlorophyll fluorescence quenching parameters in AC and $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR were also recorded 25 days after anthesis in the first year, as well as 4 days after ear emergence and 7-9 days after anthesis in the second year. Four days after ear emergence, EC did not affect maximal photochemical efficiency (F_v/F_m) measured after a 20 min dark period (Fig. 5 C) nor ϕ_{II} (Fig. 5 F), although it decreased the fraction of open PSII centres (qP), because the efficiency in excitation capture by open PSII centres (F_v'/F_m') increased, at least in plants with a high nitrogen supply. EC had no effect on F_v/F_m 7-9 days after anthesis in the second year

(Fig. 5 D), but decreased it in the first year, in later measurements during growth (25 days after anthesis, Fig. 5 B). Also after anthesis, elevated CO₂ decreased F_v'/F_m' in both years (Fig. 5 K and M), and qP in the first year (25 days after anthesis, Fig. 5 H), so that Φ_{II} was decreased in both experiments (Fig. 5 E and G). Four days after ear emergence (second year) T+4 decreased qP (Fig. 5 I), although this caused no significant changes in Φ_{II} . On this date, T+4 had no effects on other quenching parameters. Seven-nine days after anthesis, T+4 decreased F_v'/F_m' in plants with LN, but not with HN in the second experiment (Fig. 5 D), and tended to decrease it in all cases 25 days after anthesis in the first experiment ($P < 0.1$, Fig. 5 B). Late in leaf growth in the first year (25 days after anthesis), T+4 had variable effects on F_v'/F_m' and Φ_{II} depending on variety and growth CO₂, increasing these parameters in Rinconada under EC and in Alcazar with AC, while decreasing them in Alcazar under EC (Fig. 5 E and K). HN increased F_v'/F_m' 4 days after ear emergence (Fig. 5 C) and, in plants grown at T+4, also 7-9 days after anthesis (Fig. 5 D).

Discussion

Although flag leaf photosynthesis increased in response to EC, measurements at the same CO₂ concentration in plants grown at AC and EC reveal that photosynthesis was down regulated by an increase in air CO₂ levels. This occurred in well fertilized wheat crops with high leaf nitrogen content (Sánchez de la Puente et al 2000) and growing on field soil without root restrictions limiting the response to elevated CO₂ (Arp 1991). In agreement with previous results (Bunce 2001, Lodge et al. 2001), CO₂ enrichment caused a negative acclimation of stomatal conductance, as evidenced by reciprocal measurements in both EC and AC plants. However, down regulation of photosynthesis at EC was not accounted for by depressions in stomatal conductance and intercellular CO₂ concentration (Fig. 4), but by decreases in total in vitro activity and/or activation of Rubisco in leaves sampled under the growth conditions (Pérez et al. 2004). This result is in agreement with earlier reports (McKee & Woodward 1994; Sage 1994; Jacob, Greitner & Drake 1995), but in contrast with several field experiments with abundant nitrogen supply where no decrease in Rubisco has been observed (Curtis 1996; Rogers et al. 1998; Farage et al. 1998). Our data on nitrogen contents and Rubisco amount, activity and mRNA levels (Pérez et al. 2004) suggest that acclimation to elevated CO₂ was due to changes in the plant nitrogen status, rather than sugar repression of genes for Rubisco and other proteins (Geiger et al 1999).

The results show an interaction of CO₂, temperature and nitrogen over photosynthesis. Acclimation of photosynthesis after long exposure to elevated CO₂ was consistently decreased in plants grown at 4 °C above ambient temperatures and abundant nitrogen. The decrease in photorespiration and consequent displacement towards a higher temperature optimum for photosynthesis measured at elevated CO₂ (Long 1991) is not enough to account for this result, firstly because the positive interaction of higher temperatures and elevated measurement CO₂ was found in plants grown at EC, but only occasionally in AC plants; and secondly because this interaction was also observed in measurements under photorespiratory conditions. At saturating light and AC, and probably also at EC - due to the decreased Rubisco activity under CO₂ enrichment -, this effect should be due to an increase in Rubisco activity (Stitt & Schulze 1994). However, 2 days after the start of ear emergence in the second year, EC decreased in vitro initial and total Rubisco activities in leaves sampled under the growth conditions in both T and T+4 (Pérez et al. 2004).

Therefore, under the light saturated conditions of photosynthesis measurement, EC—which increased in vitro Rubisco activation relative to AC plants (Pérez et al. 2004)—combined with T+4 must have kept a high Rubisco activation that partly compensated for the decrease in total Rubisco activity. Following transfers between ambient and elevated CO₂, rapid changes in Rubisco activation have been found (Sage et al. 1988). A smaller loss of Rubisco from ear emergence to 9 days after anthesis (Pérez et al. 2004) was added to the higher Rubisco activation to account for the positive interaction of EC and T+4 with HN on the later date. Thus, at high nitrogen supply, above ambient temperatures decreased photosynthesis acclimation to CO₂ enrichment by increasing Rubisco activation initially and both activation and total activity in older leaves. To our knowledge, this interactive effect of elevated CO₂ and temperatures above those prevailing in a Mediterranean climate, at high nitrogen supply, has not been reported before.

Decreased quantum yield of PSII at high measurement CO₂ and limiting light in the first experiment, and at moderate irradiance and ambient CO₂ in both experiments after anthesis, indicates that EC decreased not only Rubisco activity, but also photosynthetic electron transport. Although the fraction of open PSII centres (qP) was decreased by EC at some sampling times, it was the efficiency in excitation capture by open PSII centres (F_v'/F_m') that was inhibited after anthesis in both years. Therefore, rising CO₂ levels affected the functioning of PSII and not only the capacity of downstream processes to accept electrons, in contrast with the results reported by Habash et al. (1995) and Hymus et al. (1999), but in agreement with Spunda et al. (1998) and, under some conditions, with Hymus et al. (2001). The depression of F_v'/F_m' in EC increases the probability of excitation energy being dissipated through non-photochemical processes in the antenna of PSII and may increase the risk of photoinhibition (Hymus et al. 2001). Decreased F_v/F_m in EC, observed 25 days after anthesis in the first experiment, may be indicative of photoinhibition, although an association with zeaxanthin-dependent quenching remaining after the 20 min dark adaptation cannot be excluded. No significant CO₂-temperature interaction was observed over fluorescence parameters, supporting the assertion that Rubisco changes were involved in the positive response of photosynthesis to the EC and T+4 combination. In contrast with EC, T+4 had no effect on the quantum yield of electron transport at low light and high CO₂ at anthesis, although at later growth stages warm temperatures decreased maximal photochemical efficiency (F_v/F_m), as

found by Scarascia-Mugnozza et al. (1996) and Roden and Ball (1996 b). Absence of this effect in the second year with high nitrogen, which delays leaf senescence, suggests that the temperature effect is attributable to accelerated ageing. The variety- and CO₂-dependent degree of senescence could account for the variable effects of temperature on : II 25 days after anthesis in the first experiment. High nitrogen has been shown to enhance the light saturated linear electron flow through Photosystem II, and to increase the quantum yield of PSII electron transport at high CO₂ - which increased the maximal Rubisco catalyzed carboxylation rate -, but not at low CO₂ (Hymus et al. 2001). In our experiments, increased F_v/F_m with high nitrogen points to an increased investment of this nutrient in components of the photochemical apparatus, although in the light nitrogen supply, which only occasionally increased Rubisco activity (Pérez et al. 2004), had no effect on : II.

In conclusion, rising CO₂ concentrations decrease stomatal conductance, although photosynthesis acclimation is due to reduced Rubisco activity rather than limited stomatal conductance. Light saturated photosynthesis in plants grown under elevated CO₂ and warm temperatures experience a lower downward acclimation associated with increases in Rubisco activity, when nitrogen supply is ample. Photochemical efficiency, and not just the fraction of open PSII centres, can be decreased by elevated CO₂ along with Rubisco-limited carboxylation.

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Figure legends

Fig. 1. Photosynthetic carbon assimilation measured at 360 (360) and 700 $\mu\text{mol mol}^{-1}$ CO_2 (700) of flag leaves of wheat grown in 360 (AC) or 700 $\mu\text{mol mol}^{-1}$ CO_2 (EC), ambient (T, white columns) or ambient + 4 °C (T+4, black columns) temperatures and (second experiment) 80 kg ha^{-1} (LN) or 120 kg ha^{-1} of nitrogen (HN). Measurements at the beginning of anthesis in the first experiment (A), and 2 days after the start of ear emergence (B) and 9 days after anthesis (C) in the second experiment. Results for wheat varieties Alcazar and Rinconada in the first experiment have been averaged. Vertical bars represent least significant differences (LSD, $P < 0.05$) for main effects of CO_2 (a), temperature and nitrogen (second year) (b). LSD for treatment interactions are omitted for clarity.

Fig. 2. Stomatal conductance measured at 360 and 700 $\mu\text{mol mol}^{-1}$ CO_2 of flag leaves of wheat grown in 360 or 700 $\mu\text{mol mol}^{-1}$ CO_2 , ambient or ambient + 4 °C temperatures and (second experiment) 80 kg ha^{-1} or 120 kg ha^{-1} of nitrogen. Symbols as in fig. 1.

Fig. 3. Concentration of CO_2 in the intercellular leaf spaces measured at 360 and 700 $\mu\text{mol mol}^{-1}$ CO_2 of flag leaves of wheat grown in 360 or 700 $\mu\text{mol mol}^{-1}$ CO_2 , ambient or ambient + 4 °C temperatures and (second experiment) 80 kg ha^{-1} or 120 kg ha^{-1} of nitrogen. Symbols as in fig. 1.

Fig. 4. Relationships between photosynthetic carbon assimilation (A) and intercellular CO_2 concentration (C_i) measured at 360 and 700 $\mu\text{mol mol}^{-1}$ air CO_2 concentrations of flag leaves of wheat grown in 360 $\mu\text{mol mol}^{-1}$ CO_2 (open symbols) or 700 $\mu\text{mol mol}^{-1}$ CO_2 (closed symbols), ambient (circles) or ambient + 4 °C (squares) temperatures and (second experiment) 80 kg ha^{-1} (B, D) or 120 kg ha^{-1} of nitrogen (C, E). Measurements at the beginning of anthesis in the first experiment (A), and 2 days after the start of ear emergence (B, C) and 9 days after anthesis (D, E) in the second experiment. Results for wheat varieties Alcazar and Rinconada in the first year (A) have been averaged. Vertical bars represent least significant differences (LSD, $P < 0.05$) for main effects of CO_2 (a), temperature and nitrogen (second year) (b). LSD for treatment interactions are omitted for clarity.

Fig. 5. Chlorophyll a fluorescence parameters of flag leaves of wheat cultivars Alcazar (Alc) and Rinconada (Rin) (first experiment) or Alcazar (second experiment) grown in 360 or 700 $\mu\text{mol mol}^{-1}$ CO_2 , ambient or ambient + 4 °C temperatures and (second experiment) 80 kg ha^{-1} or 120 kg ha^{-1} of nitrogen. Measurements at 11 (A) and 25 (B, E, H, K) days after the beginning of anthesis in the first experiment and at 4 days after the start of ear emergence (C, F, I, L) and 7-9 days after anthesis (D, G, J, M) in the second experiment. Symbols as in fig. 1.

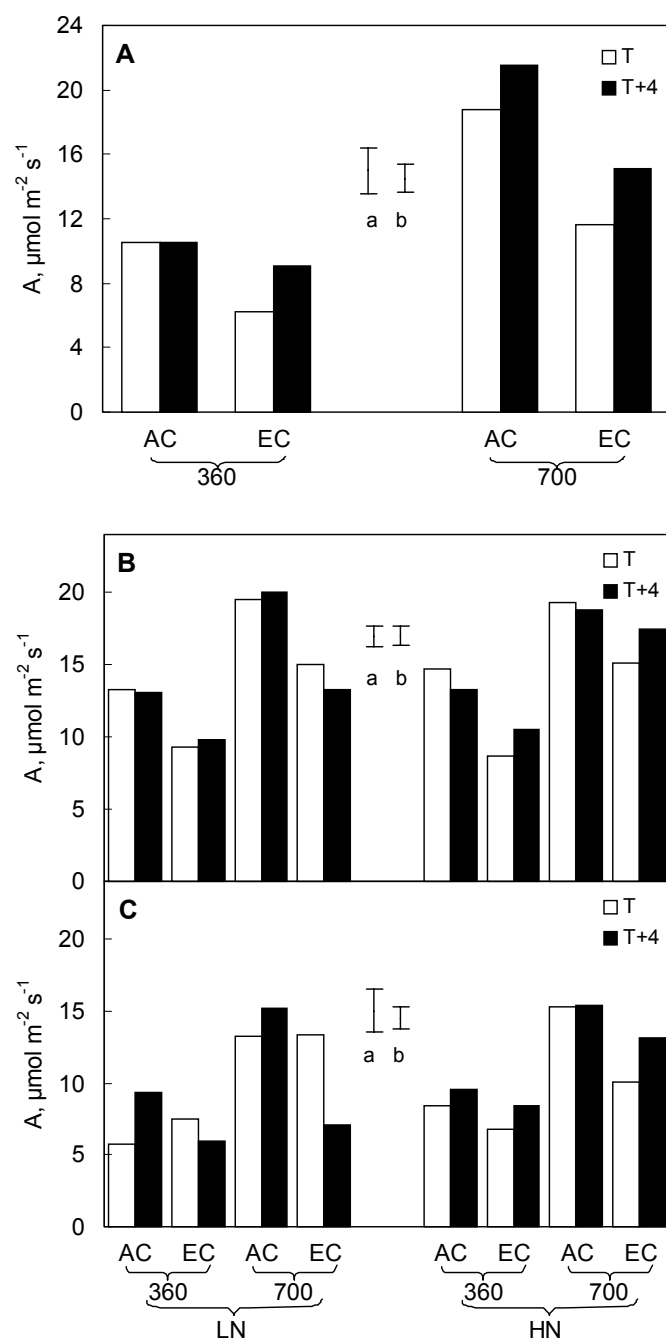


Fig. 1

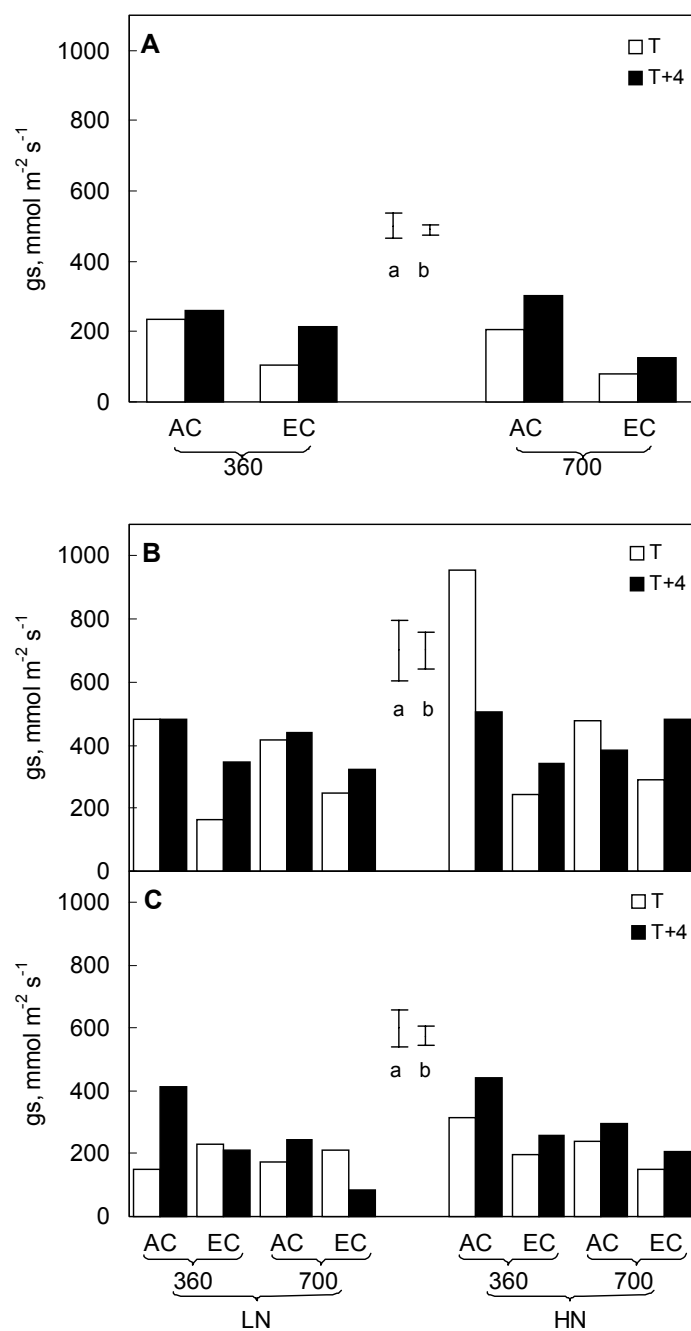


Fig. 2

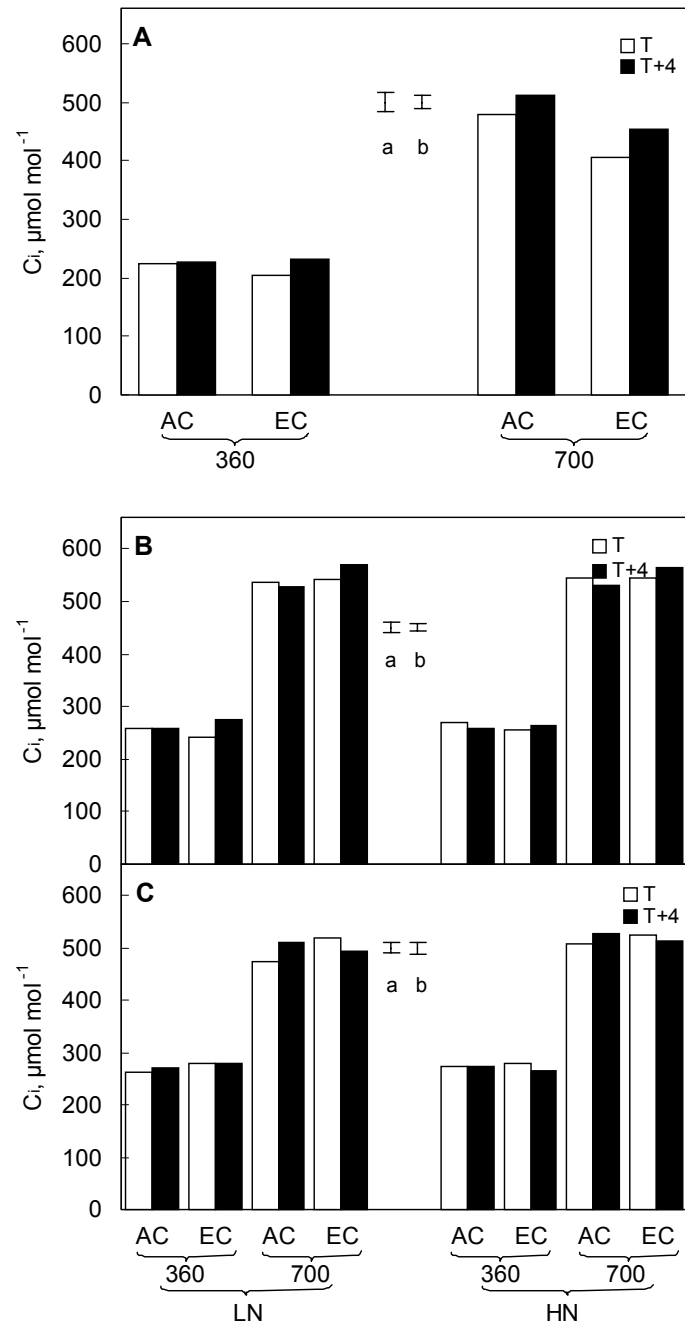


Fig. 3

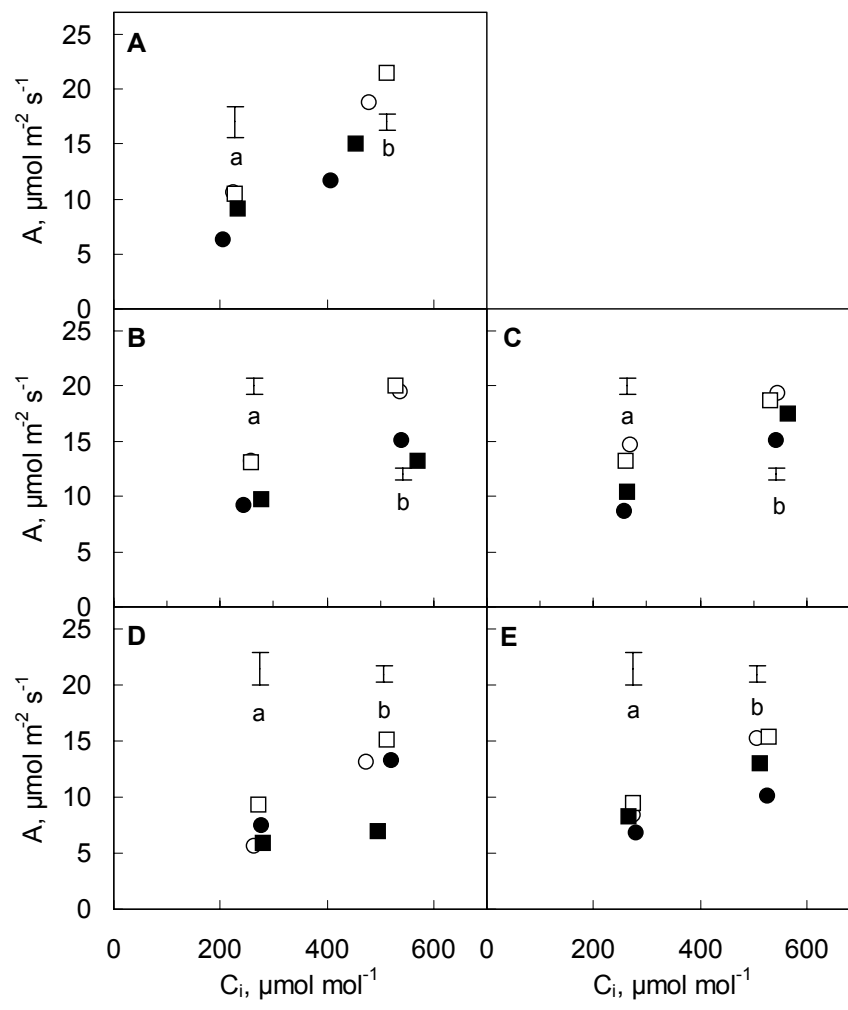


Fig. 4

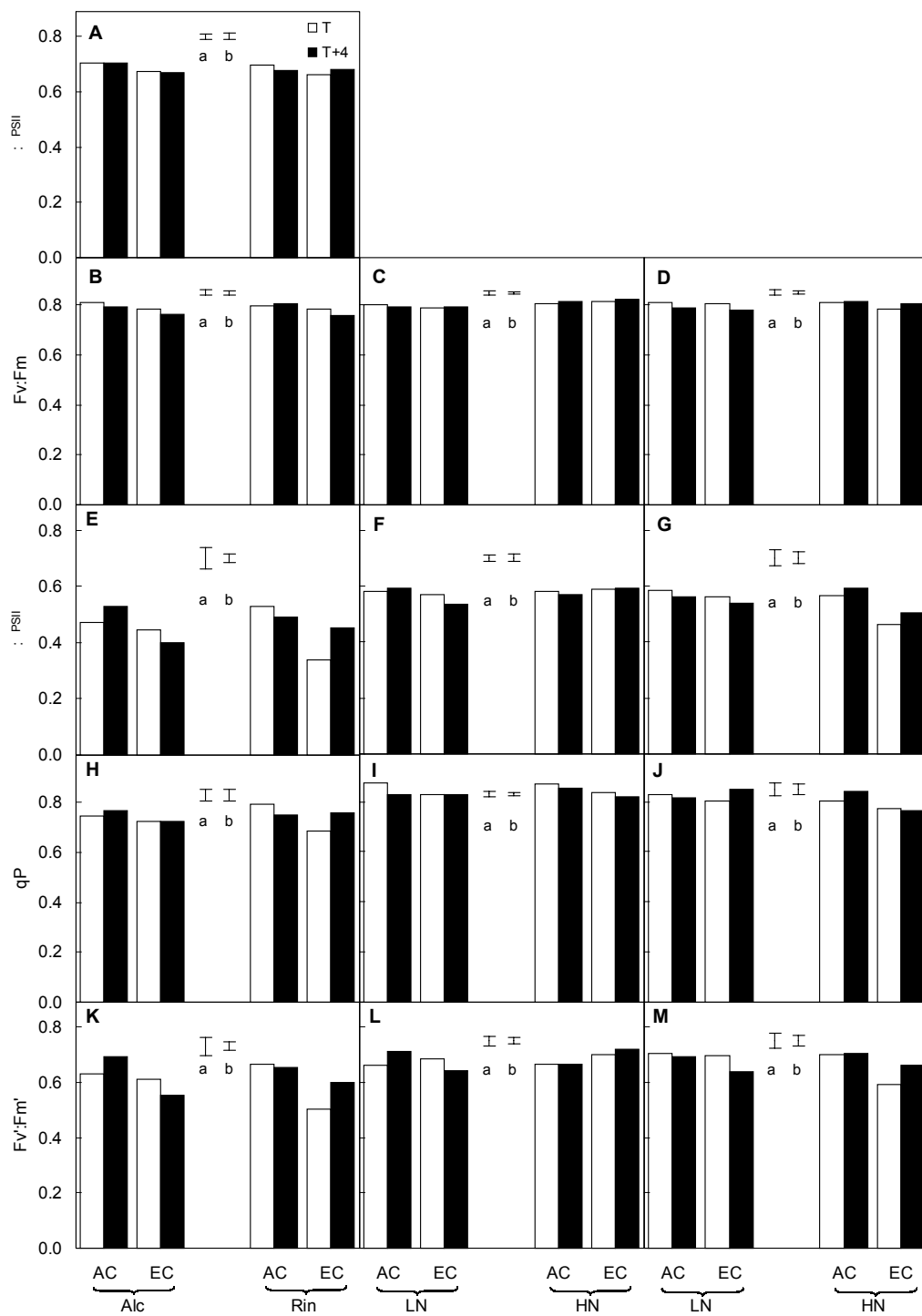


Fig. 5